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(54) **TRANSGENIC LEGUMES**

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**C12N 15/82** (2006.01)

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**(58) Field of Classification Search**

None

See application file for complete search history.

**(56) References Cited****U.S. PATENT DOCUMENTS**

5,665,892 A *	9/1997	Van Assche .....	C07K 16/40
			435/320.1
5,714,365 A	2/1998	Van Assche et al.	
5,767,365 A	6/1998	Sonnewald	
6,107,547 A	8/2000	Coruzzi et al.	
6,288,240 B1	9/2001	Martinez et al.	
6,555,500 B1	4/2003	Unkefer et al.	
6,593,275 B1	7/2003	Unkefer et al.	
RE38,446 E	2/2004	Van Assche et al.	
6,723,898 B2	4/2004	Sonnewald	
6,756,218 B2	6/2004	Allen et al.	
6,831,040 B1	12/2004	Unkefer et al.	
6,864,405 B1	3/2005	Coruzzi et al.	
7,091,400 B2	8/2006	Haigler et al.	
7,176,009 B2	2/2007	Allen et al.	
7,989,677 B2	8/2011	Tanksley et al.	
8,551,917 B2	10/2013	Unkefer et al.	
2004/0077090 A1	4/2004	Short	
2005/0124010 A1	6/2005	Short et al.	
2007/0218556 A1	9/2007	Harris et al.	
2010/0115662 A1	5/2010	Gupta et al.	
2010/0170009 A1	7/2010	Unkefer et al.	
2010/0186121 A1	7/2010	Unkefer et al.	
2010/0263090 A1	10/2010	Unkefer et al.	
2011/0004961 A1	1/2011	Unkefer et al.	
2011/0030089 A1	2/2011	Unkefer et al.	

2012/0060235 A1	3/2012	Privat et al.
2012/0144528 A1	6/2012	Unkefer et al.
2013/0160158 A1	6/2013	Gupta et al.
2013/0232641 A1	9/2013	Unkefer et al.
2013/0239256 A1	9/2013	Unkefer et al.
2014/0038824 A1	2/2014	Unkefer et al.

**FOREIGN PATENT DOCUMENTS**

WO 9216631	10/1992	
WO 2011025515 A1	3/2011	
WO 2011106794 A1	9/2011	
WO 2012134906 A1	10/2012	

**OTHER PUBLICATIONS**

- Gallais et al, 2005, Maydica, 50: 531-547.\*  
Foyer et al, 1994, Biochemical Society Transactions, 22: 909-915.\*  
Fei, et al., "Overexpression of a soybean cystolic glutamine synthetase gene linked to organi-specific promoters in pea plants grown in difference concentrations of nitrate", Planta, 2003, 467-474.  
Hirel, et al., "Forcing expression of a soybean root glutamine synthetase gene in tobacco leaves induces a native gene encoding cytosolic enzyme", Plant Molecular Biology, 1992, 207-218.  
Laporte, et al., "Promoter strength and tissue specificity effects on growth of tomato plants transformed with maize sucrose-phosphate synthase", Planta, 2001, 817=822.  
Luis-Ortega, et al., "Regulatory Mechanisms Underlying Post-transcriptional Regulation of Cytosolic Glutamine Synthetase in Alfalfa", Poster, 2009.  
Miao, et al., "Ammonia-Regulated Expression of a Soybean Gene Encoding Cytosolic Glutamine Synthetase in Transgenic Lotus corniculatus", The Plant Cell, 1991, vol. 3, 11-22.  
Ortega, et al., "The 3' untranslated region of a soybean cytosolic glutamine synthetase (GS1) affects transcript stability and protein accumulation in transgenic alfalfa", The Plant Journal, 2006, 832-846.  
Seger, "Manipulation of sucrose phosphate synthase (SPS) activity in *Medicago sativa* (Alfalfa)", [http://www.nmspacegrant.com/files/tiny\\_mee/file\\_manager/fellowships\\_research/MarkSeger-2009.pdf](http://www.nmspacegrant.com/files/tiny_mee/file_manager/fellowships_research/MarkSeger-2009.pdf), Oct. 23, 2009.

\* cited by examiner

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**(57) ABSTRACT**

Embodiments of the present invention comprise altering the biosynthesis and accumulation of sucrose in legumes by transforming the plants with the sucrose phosphate synthase (SPS) gene of maize, and closely related regulatory genes. Embodiments of the present invention further comprise altering the assimilation of nitrogen in legumes by transforming the plants with the glutamine synthetase (GS) gene of soybean, and closely related regulatory genes. Embodiments of the present invention further comprise transforming legume plants with both SPS and GS genes. In addition, embodiments of the present invention relate to enhancing expression of transgenes through the 5' UTR of the *glycine max* (soybean) cytosolic glutamine synthetase (*Gmglnβ1*) gene.

**4 Claims, 38 Drawing Sheets**